'Eureka' Lemon Chilling Injury

Steven J.R. Underhill, Richard L. McLauchlan, and Irving L. Eaks[1]
Horticulture Postharvest Group, Queensland Department of Primary Industries, 19 Hercules Street, Hamilton, Queensland 4007, Australia

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Abstract. **In accordance with the currently approved Australian citrus disinfestation protocol for export to Japan, degreened 'Eureka' lemons [*Citrus limon* (L.) Burm.] were cold-stored for 2 weeks at 1C. Following cold treatment, fruit were stored at 5C for 3 weeks, then transferred to 20C for an additional week to simulate transportation and handling. Fruit harvested early in the season were more susceptible to chilling injury than fruit harvested later, with 62% having lesions >1 cm^2 after 2 weeks at 1C. Most of the chilling injury occurred after subsequent storage (at 5C) rather than immediately after the 1C treatment. Injury was different from surface pitting or oleocellosis, manifesting as large uniform surface lesions 2 to 3 cm in diameter that rapidly discolored following storage at 20C. Although the oil glands were flattened, the collenchyma layer immediately above the oil gland remained intact. Cellular discoloration was localized around the oil gland, possibly indicating a lateral release of oil gland contents. Nondegreened late-season fruit developed substantially lower levels of chilling injury.**

Cold disinfestation of Australian lemons (*Citrus limon* cv. Eureka and Lisbon) at 1C for 14 days (Hill et al., 1988) was recently accepted by the Japanese Ministry of Agriculture, Forestry, and Fisheries as a quarantine treatment for Queensland fruit fly. In response to the withdrawal of ethylene dibromide registration, similar disinfestation treatments also have been investigated for other citrus fruit (Adsule et al., 1984, 1987; Chalutz et al., 1985; Hill et al., 1988; Houck et al., 1990; Purvis, 1984). The first sea-freight consignment of cold-disinfested 'Eureka' lemons from the Gayndah region (southeastern Queensland, Australia) was exported to Japan in May 1992. On arrival, 60% to 70% of the fruit showed rind breakdown. Injury was characterized as large sunken lesions in the peel, which rapidly discolored once fruit were returned to ambient temperature.

McLauchlan et al. (1989) previously reported no chilling injury (CI), oleocellosis, or any other surface defects of late-season Queensland 'Eureka' lemons during 18 days of storage at 1C. However, the 1992 export fruit were harvested several weeks earlier in the season and were degreened with ethylene before the cold-disinfestation treatment. Although no further shipments were sent to Japan in 1992, export of nondegreened (silvergreen grade) 'Lisbon' lemons from the Mildura region (Victoria, Australia) in the following year (mid-season harvest) arrived in excellent condition (B. Tugwell, personal communication).

Our experiment investigated the importance of harvest date and fruit degreening on the incidence of CI in 'Eureka' lemons. As the observed surface lesions differed from previously documented CI, the anatomical characteristics of the injury were examined.

Materials and Methods

Mature 'Eureka' lemon fruit were harvested weekly from two commercial orchards in Gayndah (lat. 25°S, 300 km from Brisbane), Queensland, Australia, from 16 Apr. through 4 June 1993. Fruit were degreened with 5 to 10 ppm ethylene for 1 to 2 days at 27C. Nondegreened fruit also were sampled at harvests 6 to 8 and were held at ambient temperature while the other fruit were degreened. All fruit then were treated with 2-(4-thiazolyl) benzimidazole [thiabendazole (TBZ)] (TBZ: 500 ppm a.i.), coated with Superglo wax (Peerless Emulsion, Brisbane, Queensland, Australia), size-graded, packaged, and road-transported to the Hamilton laboratories, Brisbane, within 48 h. A minimum of six cartons (30 liters, 138 to 162 fruit) from each of two growers (separate packinghouses) were obtained at each harvest date.

Cartons were forced-air cooled overnight in a cool room operating at 0.5 ± 0.3C, with pulp temperature reduced to 1C after 8 h. The dewpoint of the cool room (–1.5C) and fruit pulp temperatures were monitored using a dewpoint monitor (Hydro-M2; General East-

Table 1. Effects of harvest date, grower, and removal time–temperature on the incidence of chilling injury.

Source	df	Mean square
Harvest (H)	7	2114.26*
Grower (G)	1	3895.89
Error (HG)	7	343.95
Removal (R)	2	4691.62**
H × R	14	68.99
Error (HGR)	10	140.36

*,**Significant at $P \leq 0.05$ or 0.01, respectively.

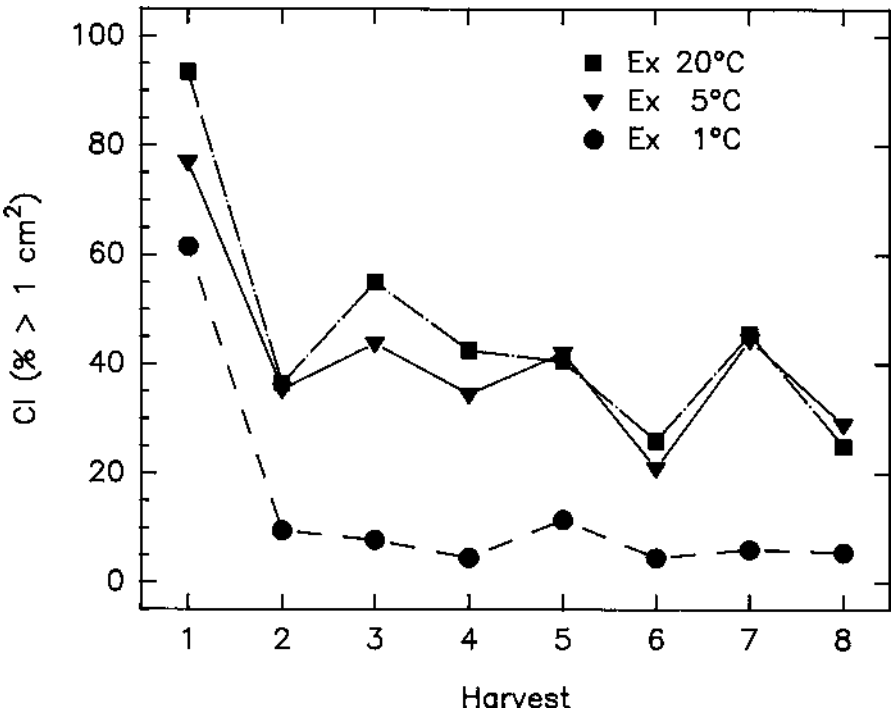

Fig. 1. Incidence (percentage of fruit with >1 cm^2 of surface area affected) of chilling during weekly harvests throughout the season. Fruit were degreened and stored for 2 weeks at 1C, 3 weeks at 5C, and 1 week at 20C.

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[1]Current address: Dept. of Biochemistry, Univ. of California, Riverside, CA 92521.

ern Instruments, Watertown, Mass.). After 2 weeks at 1C, fruit were transferred to 5C and held there for 3 weeks, after which they were held at 20C for an additional week.

The incidence of surface CI was determined qualitatively. Ten fruit per layer (50 per carton) were randomly selected from each carton. Two staff members estimated the total surface area injured (sunken lesions or discolorations) to the nearest 0.25 cm^2 by comparing the area to an area template. CI was defined as the percentage of fruit having >1 cm^2 surface area injured. At the end of each storage period, fruit were repacked into the same cartons. Results were analyzed by a balanced, factorial, split-plot analysis of variance, with significant differences calculated by protected least squares method using the DPI-BALF (Biometry, Queensland Dept. of Primary Industries, Queensland, Australia) program.

Pericarp anatomy was assessed progressively during fruit storage using a light microscope (model BH-2; Olympus, Tokyo). Pericarp samples (2 × 2 mm) were fixed with formalin-aceto-alcohol (90 ml 70% ethanol, 5 ml glacial acetic acid, and 5 ml formalin) and were dehydrated using an ethanol series. Samples were embedded in paraffin wax and sectioned with a microtome at 8-µm thickness. Sections were stained for lignin and cellulose distribution using safranin and fast green. To localize cellular discoloration, tissue was hand-sectioned and mounted in 30% glycerol.

Table 2. Mean daily field temperature and total rainfall for the week immediately before each harvest (16 Apr. to 4 June 1993).

	Temp (°C)		Rainfall
Harvest	Min	Max	(mm)
1	14.7 ± 2.9	29.4 ± 1.0	6
2	14.9 ± 1.8	29.5 ± 1.8	0
3	13.9 ± 2.3	27.6 ± 1.7	0
4	11.5 ± 2.0	27.3 ± 1.3	4
5	14.3 ± 2.5	27.4 ± 1.8	5
6	13.4 ± 3.2	25.6 ± 1.0	0
7	11.6 ± 1.0	26.2 ± 1.6	16
8	14.5 ± 1.5	24.2 ± 1.3	0

Results and Discussion

Fruit stored at 1C for 2 weeks developed moderate CI levels (5% to 12%) (Fig.1, Table1), except at harvest 1 when 62% of fruit developed lesions >1 cm^2. Incidence of CI in harvest 1 fruit increased to 77% and 94% following subsequent storage at 5C and 20C, respectively. CI varied with harvest date; early season fruit (harvest 1) were the most susceptible to surface damage. The CI level was lowered after harvest 1, with no significant difference in CI incidence at subsequent harvest dates. Most CI was not present immediately after the 1C treatment but after the subsequent 5C storage period. The minor increase in CI during the final week at 20C was due to the injury becoming more prominent from discoloration of surface lesions.

A correlation between harvest date and susceptibility to CI has been reported in lemons by Houck et al. (1990). Tugwell and Nechvoglod (1987) also reported a seasonal increase in surface blemish. Seasonality also has a significant effect on CI susceptibility in 'Marsh' grapefruit (*Citrus paradisi* Macfad.) (Benschoter, 1979; Davis, 1973). Kawada et al. (1978) reported that susceptibility to CI was related to minium field temperature, with higher temperatures resulting in increased CI resistance. However, Houck et al. (1990) suggested that early season (high temperature) fruit was more susceptible to CI than mid- or late-season (lower temperature) fruit. Resistance has been related to accumulation of reducing sugars (Purvis and Grierson, 1982), proline content (Purvis, 1981), and putrescine concentration (McDonald and Kushad, 1986) in the peel.

Table 3. Effect of harvest time (harvest 6, 7, or 8 only), grower, ethylene, and initial temperature on chilling injury.

Source	df	Mean square
Harvest (H)	2	97.1
Grower (G)	1	816.7*
Error (HG)	2	31.3
Temperature (T)	1	1410.7*
H × T	2	35.3
Ethylene (E)	1	1700.2**
H × E	2	161.3
T × E	1	748.2*
H × T × E	2	45.8
Error (HGET)	9	142.1

*, **Significant at $P \leq 0.05$ or 0.01, respectively.

CI incidence increased slightly at harvest 7 compared to harvests 6 and 8 (Fig.1). Coincidentally, daily average minimum temperature dropped, and 16 mm of rain fell in the week before harvest 7. However, the significantly higher CI at harvest 1 could not be attributed directly to field temperature or rainfall before that harvest, and we found no consistent evidence of a relationship between minium field temperature before each harvest and the subsequent CI incidence (Table 2). Similarly, differences in maximum field temperature and rainfall also were not consistent with increased CI.

Fruit degreening before storage at 1C for 2 weeks (harvests 6 to 8) increased the level of surface CI compared to nondegreened fruit (Fig. 2, Table 3). Again, the CI incidence increased following subsequent storage of fruit at 5C. Where fruit were not degreened before cold disinfestation, the CI incidence was ≤10% and, except for harvest 8, was <3%. At harvest 7, 10% of degreened fruit that were not subjected to the 1C treatment still developed unacceptable levels of CI. Nondegreened lemons not subjected to the 1C treatment developed only low levels of CI (0% to 3%). Slightly higher CI was recorded in 1C, cold-treated, nondegreened fruit, except for harvest 8 where the incidence of CI increased to 10% after transfer to 5C and then 20C.

Increased susceptibility to CI following degreening has been reported in 'Bearss' lemons by McDonald (1986) and 'Marsh' grapefruit by Hatton and Cubbedge (1981). Similarly, Cohen et al. (1983) noted that harvesting yellow 'Villa Franka' lemons resulted in a reduction of pitting injury associated with storage at 2C. Reduced CI associated with the second commercial export shipment to Japan may be due partly to the use of nondegreened fruit. However, possible difference in fruit maturity cannot be discounted.

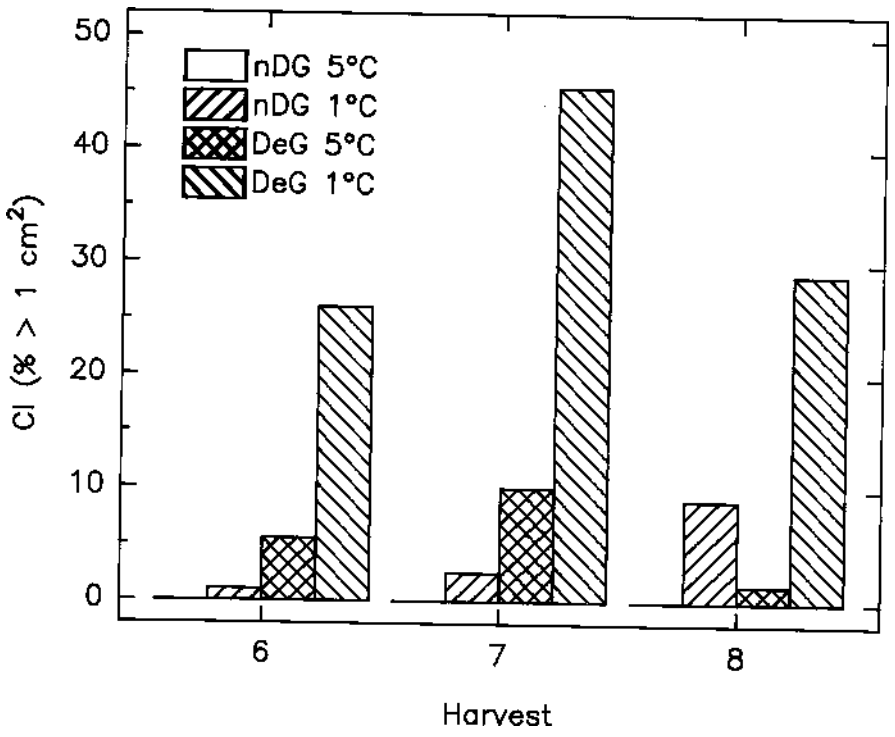

Fig. 2. The effect of degreening (DeG) vs. nondegreening (nDG) on the incidence of surface chilling symptoms of fruit initially stored at either 1 or 5C for 2 weeks (harvests 6 to 8), followed by 3 weeks at 5C and 1 week at 20C.

CI was characterized by several large sunken lesions on the peel surface (Fig. 3). Lesions were 2 to 3 cm in diameter and ≈2 mm deep. This injury differed distinctly from the surface pitting reported by Chalutz et al. (1985), Cohen et al. (1983), and Purvis (1984): the surface lesions were larger and more uniform in shape. Injury was also different from tissue damage associated with rots. Although external injury resembled damage caused by oleocellosis, the flattened oil glands remained intact and had a continuous collenchyma layer (Fig. 4a). The collapse of the oil gland and surrounding tissue (Fig. 4 b and c) did not correspond to scanning electron microscope sections of oleocellosis reported by Wild (1992). Instead, the oil gland cap was slightly concave with no evidence of increased cellular collapse in the adjacent tissue (Fig. 4a). Tissue browning was associated with localized discoloration around the oil glands, suggesting a possible lateral release of oil gland contents into the surrounding parenchyma cells.

Although there was some evidence of cellular collapse associated with the lesion, the orientation of the parenchyma cells and the presence of an intact epidermis (Fig. 4c) were consistent with damage caused by basipetal pressure. This aspect was particularly evident in the parenchymatous tissue at the margin of each lesion. Many lesions appeared to correspond to areas of fruit-to-fruit contact. However, the incidence of injury was not related to the position of the fruit in the carton, with fruit at the top of the carton displaying similar injury to that of fruit in the bottom layer.

CI was influenced by harvest date and degreening before low-temperature disinfestation. Although these procedures account for the differences in CI between two specific commercial export shipments to Japan, additional factors, such as cultivar and regional and seasonal variability, were not examined. Further work is required to determine the importance of these factors in relation to CI susceptibility.

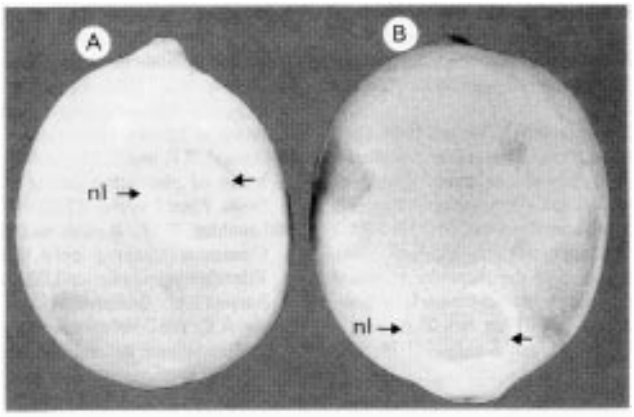

Fig. 3. Chilling injury on lemon peel. (**A**) Lesion before 20C storage; (**B**) subsequent discoloration of the lesion following storage at 20C [necrotic lesion (nl)] (×0.32).

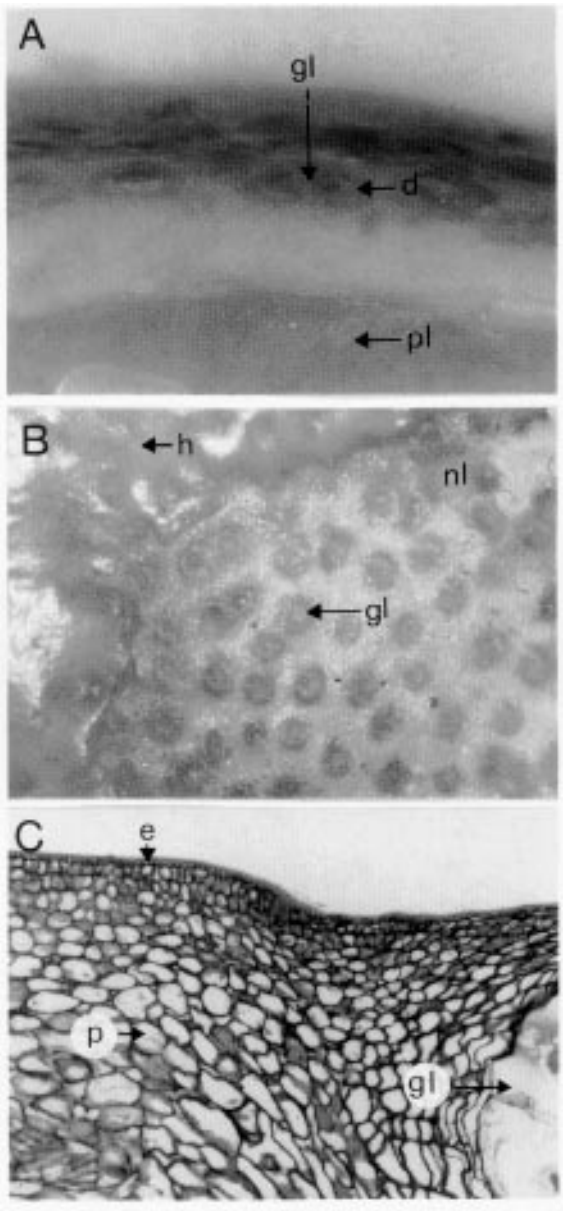

Fig. 4. Fruit injury following 2 weeks of storage at 1C, 3 weeks at 5C, and 1 week at 20C. (**A**) Cross-section of fresh-mount peel illustrating necrotic lesion, compressed oil glands, and localized tissue discoloration around the oil gland [oil gland (gl), discoloration (d), pulp (pl)]; (×29). (**B**) A magnified view of the surface of the necrotic lesion before discoloration, illustrating individual oil glands [oil glands (gl), necrotic lesion (nl), non-necrotic tissue (h)] (×6). (**C**) Light microscope section of lemon peel illustrating (**left**) the healthy tissue and (**right**) the sunken necrotic lesion [oil gland (gl), parenchyma (p), epidermis (e)] (×12).

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